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RESPONSE OF RICE PLANTS TO JASMONIC ACID RATES IN RELATION TO TIME OF APPLICATION

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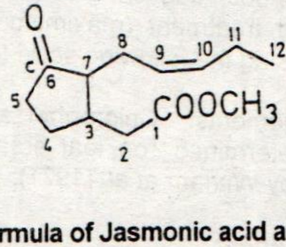
ABSTRACT

Two field experiments were conducted at the Experimental Farm of Agric. Minufiya University, during 1999 and 2000 seasons, to study the effect of Jasmonic acid (JA) rates namely i.e. 00, 500 and 1000 mg L^{-1} at two times of application on the rice development, paddy yield and grain chemical composition were investigated. It was found that JA strongly reduced plant height and increased number of tillers and main culm diameter. The higher rate of JA applied at the early time of application (stage 7) was the most retarding treatments. Spraying JA at the rate of 1000 mg L^{-1} at growth stage 7 decreased the length of the middle and upper internodes of plant culm. Early JA application at the higher level was more effective in reducing rice lodging by increasing plant shortening. JA application at growth stage 7 tended to decrease flag leaf area, and chlorophyll concentration. JA application decreased IAA and GA whereas cytokinin and ABA increased when rice plants sprayed at growth stage 7. Paddy yield and starch and protein content were increased with the lowest rates of JA at growth stage 9. N, P and K in grains were increased by JA application at growth stage 9.

INTRODUCTION

Jasmonate (JA) and its methyl ester (Me JA) belong to a group of novel plant hormones (Fig. 1) called Jasmonates (Pathier, 1991 and Staswick, 1995), and they are widely distributed in higher plants (Sembdner and Klose, 1985; Farmer *et al.,* 1992 and Constabel *et al.,* 1995), and is involved in regulation of a variety of plant functions and has a variety of biological activities (Meyer *et al.,* 1984 and Staswick, 1995). They cause growth inhibition (Yamane *et al.,* 1981; Miersch *et al.,* 1986 and Popova *et al.,* 1988), and non-toxic inhibition of pollen germination is caused by JA, especially in high concentration (Sembdner and Parthier, 1993 and Creelman and Mullet, 1995). Jasmonates are known to have several biological activities, including promoting stomatal closure and accelerating leaf senescence in oats and barly (Weidhase *et al.,* 1987), apparently mediating their effect by controlling gene expression, inducing of tuberization in potato (Koda *et al.,* 1991).



R = H, Jasmonic acid **R = CH_3, Methyl Jasmonate.**

Fig. (1): Structures formula of Jasmonic acid and methyl ester (According to Vick and Zimmermann, 1986).

Rice is one of the main cereal crops in Egypt. Recently it became the second exported crop after cotton. It is also the most economic one to be planted under some plant growth inhibitors. Rice cover about 16.9% of the cultivated area. The northern part of the Nile Delta is the main belt; it includes 95% of the rice cultivated area.

Lodging is a main problem in the intensive rice cropping with high N-application rates because it greatly influences the time of harvest and decrease yield. Lodging reduced rice yields about 30-40% (Basak, 1962).

The objective of this research was to examine the effect of Jasmonic acid (JA) on the growth, paddy yield and chemical composition of rice grains and to find out the possibility of increasing the resistance of rice plants to lodging.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Research Station in the Faculty of Agriculture Shibin El-Kom, Minufiya University during 1999 and 2000 seasons, to study the effect of Jasmonic acid (JA) levels on growth, yield and its components as well as chemical composition of rice. Rice grains (*Oryza sativa* L.) cv. Giza 172, (a long stem variety that characterized by high rate of lodging) were sown in May 25th in both seasons. Thirty five days later, seedlings were transplanted at 20 × 20 cm spacing in a complete randomized block design with four replications.

JA (Jasmonic acid was kindely obtained from Prof. Dr. Miersch at Plant Biochemistry, Martin Lather University, D. Giessen, Germany) was applied as sprays at the rate of 500 and 1000 mg L^{-1}, besides the distilled water as a control, containing 0.1% of Tween 20 as surfactant, was applied to run off onto the foliage of the plants, using a hand held manual atomizer. JA was sprayed at two times of applications. The first application was when the plants had the 1st node of main culm (growth stage, 7) and the second one at the growth stage, 9 (two weeks before heading) (Zadoks, 1974).

Fertilization and other agricultural practices were done as recommended by the Ministry of Agriculture. The following data were recorded:

Growth characters, such as plant height, internodes length, number of tillers, culm diameter (mm) was determined at the third internode from the bottom, dry weights of shoots, flag leaf area (m^2/m^2 Land) was estimated at 15 days after the second treatment (maximum length × maximum width × 0.72). Degree of lodging using the following scale (7 = no lodging and 9 = total lodging).

Photosynthetic pigments: Chlorophyll a, b and carotenoids were spectrophotometrically determined on leaf area (mg/dm^2) (15 days after treatments) as described by Witham *et al.* (1971).

Endogenous phytohormones:

The phytohormones such as, IAA, GA, cytokinin and ABA were determined using GLC. The type and amount of endogenous GA-like substances were determined by homogenizing ten gms fresh weight of rice leaves collected from each treatment (15 days after treatments), in 100% methanol, extracting overnight, and filtering the slurrvy. The supernatant was evaporated to the aqueous phase, acidified to pH 3 and eluted from a charcoal : celite (1 : 1, w / w) column with 80% acetone. After the elute was evaporated to the aqueous phase and acidified to pH 2.5, the GA like substances were partitioned into ethyl acetate, concentrated, partitioned into 1 M potassium phosphate buffer (pH 7), acidified to pH 2.5 and again partitioned into ethyl acetate (Jones *et al.*, 1980), and was reduced in volume to be used for GLC determination.

The dried basic ethyl acetate fraction was dissolved in 80% methanol. The methanol was evaporated under vacum, leaving an aqueous phase which was adjusted to pH 2.8 with 1% HCl and portioned three times with 25-50 ml of ethyl acetate. The ethyl acetate phase was reduced to 5 ml volume and stored in fridge until GLC analysis for neutral auxins. The remaining aqueous phase was adjusted to pH 5.5 with 1% NaOH and partitioned three times with 50 – 100 ml of water saturated butanol. All butanol phases were combined and reduced to 5 ml volume and used for GLC determination of acidic hormones such as ABA, and cytokinin (Mac Millan, 1970).

Mineral composition:

Total nitrogen (N) was determined by the micro-kjeldahl method. Phosphorus (P) and potassium (K) were determined in dry rice shoots as described by A.O.A.C. (1975).

At harvest stage:

The grain yield/fed. straw yield/feddan; number of panicles/m^2, the weight of 1000 grains in grams were determined.

The grain samples were subjected to chemical analysis for N-estimation using the microkjeldahl technique as described by Ling (1963). Protein values was calculated by multiplying the N value by the conversion factor 6.25. P and K in rice grains were determined as described by A.O.A.C. (1975). Starch percentage in rice grain was determined using the optical rotation measurement as described by Bassler and Putuka (1970).

All data were statistically analyzed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Growth characters:

The effect of JA on growth parameters are shown in Table (1). A gradual decrease in plant height with increasing JA level, differences were significant with the exception of JA at 500 mg L^{-1} on growth stage 9. Application JA at 1000 mg L^{-1} rate on growth stage 7, decreased the length of the middle and upper internodes of plant culm. However, it did not affect on the length of lower internodes (Fig. 2).

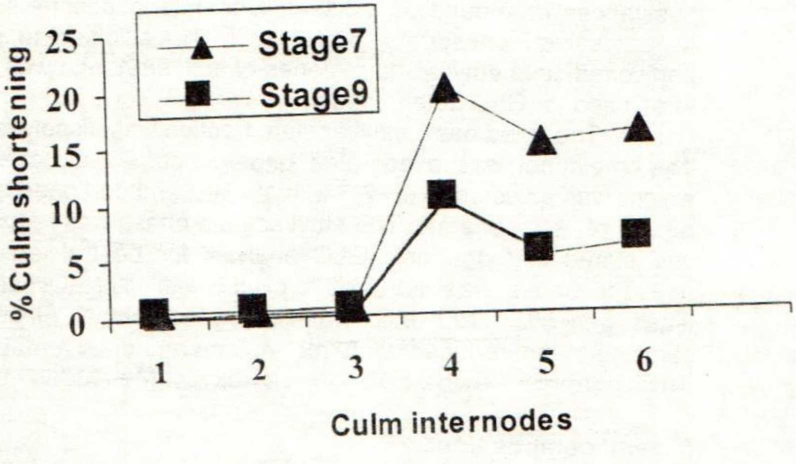


Fig. (2): Effect of JA (1000 mg L^{-1}) on internode shortening in growth stage 7 and 9.

Early application of JA had a significant effect on growth parameters as demonstrated in Table (1). Results indicated a significant depression of the stem and its internodes elongation with all JA levels, in case of early application. Similar results were obtained by Gendy and Selim (1994) and Ueda *et al.* (1994).

Table(1): Effect of Jasmonic acid on the growth characters of rice plants at harvest stage (combined data of 1999 and 2000 seasons).

JA levels $mg.L^{-1}$	Growth stage	Plant height cm	No. of tillers/m^2	Culm diameter mm	Shoot D.W. Kg/m^2	Flag leaf area\m^2	Degree of lodging*
Control		148.2	442.6	4.0	1.30	130.2	8
500	7	134.4	450.2	4.4	1.52	139.8	6
1000	7	122.8	462.4	4.8	1.64	142.8	3
500	9	140.5	444.8	4.2	1.42	140.2	7
1000	9	130.2	456.8	4.6	1.60	141.5	4
L.S.D 5%		15.2	37.2	0.4	0.07	3.6	

Degree of lodging using the following scale * 7= No lodging 9= total lodging

The overall effect of JA on the number of tillers, culm diameter and dry weight of shoots, showed mostly a significant increase if compared with untreated plants. This may be attributed to the inhibiting effects of JA on the apical dominance as a result of reducing auxin transport to apical merestims thus stimulating the growth of basal buds leading to a subsequent release of more tillers/plant and consequently, increasing their number/m^2. These results are in agreement with those reported by Mierch *et al.* (1986); Abe *et al.* (1990); Matsuki *et al.* (1992) and Takahashi *et al.* (1994 and 1995). These results suggest that in contrast to GA and IAA, which promote cell elongation, Me JA stimulates the expansion of plant cells. Me JA can also increase the respiration of plants (Cao *et al.*, 1998).

Data recorded in Table (1) revealed that JA significantly stimulated the shoots dry weight. It is obvious that the stimulative effect of JA on the number of tillers and leaf area / plant as well as the increase in assimilates such as starch (Table 4) in plant tissues could contributed to the increase in dry matter content.

Degree of lodging was decreased by about of 60% at the high level of JA as compared to the control plants. JA acts as gibberellin antagonists and increased standing ability of plants, on one hand by reducing the culm length and on the other hand, by increasing the culm diameter and strengthen its wall. This result are in harmony with the finding of Abe *et al.* (1990); Matsuki *et al.* (1992) and Takahashi *et al.* (1995), who mentioned that, the mechanism underlying these morphological effects is though to be that of JA and Me JA stimulation of the expansion of some specific cells.

It can be concluded that, the early application (growth stage 7) of JA is more effective in inhibiting rice growth than late application.

Photosynthetic pigments:

Data in Table (2) showed that, both levels of JA when applied on early growth stage (stage, 7), significantly decreased both chl. a and chl. b if compared to untreated plants. On the other hand, JA at 500 mg L^{-1} when applied at late growth stage (stage, 9), significantly increased both chl. a and chl. b. As regards to carotenoids, both JA rates significantly increased carotenoids concentrations, whereas decreased chl. a+b / car ratio. For the inhibitory effect of JA on photosynthetic pigments, it was suggested that, Methyl Jasmonate caused a rapid and strong senescence (chlorophyll degradation) of rice leaves (Chen and Kao, 1998). Moreover, Mierch *et al.* (1986); Weidhase *et al.* (1987) and Hermann *et al.* (1992), found that JA induces senescence and reduce pigments content in barley leaves. Meanwhile, Omer *et al.* (2000) mentioned that, Jasmonate application did not induce senescence in Grapevines plants. Moreover, Kovac and Ravnikar (1994) reported that, JA treatment resulted in an increase of active cytokinin content, without drastic change in photosynthetic pigments.

Table (2): Effect of JA on photosynthetic pigments (mg/dm^2) of rice leaves (combined data of 1999 and 2000 seasons).

Endogenous phytohormones concentration:

JA levels mg.L^{-1}	Growth stage	Chl.a	Chl.b	Carot.	Chla+b/Car. ratio
Control		20.6	7.61	0.46	61:1
500	7	18.0	6.20	0.52	47:1
1000	7	16.4	5.40	0.58	38:1
500	9	21.8	8.65	0.50	59:1
1000	9	18.8	5.90	0.55	45:1
L.S.D 5%		1.92	0.32	0.03	_

Endogenous phytohormones concentration

Data presented in Table (3) exhibit a marked decrease in IAA and GA concentration of treated rice leaves at both JA levels if compared with untreated leaves.

Table (3): Effect of JA on endogenous phytohormones concentration (ng.g^{-1}Fwt) in rice leaves (combined data of 1999 and 2000 seasons).

JA levels mg.L^{-1}	Growth stage	Phytohormone concentration ng.g^{-1}Fwt				
		IAA	GA	Cyt	ABA	Activ./inh.
Control		122	84	220	18.0	24:1
500	7	118	66	225	19.2	21:1
1000	7	116	44	235	19.8	20:1
500	9	120	72	230	19.4	16:1
1000	9	119	53	228	20.5	20:1
L.S.D 5%		NS	8	12	0.5	_

On the contrary, cytokinin significantly increased by using 1000 mg L^{-1} at early growth stage (7) and 500 mg L^{-1} at late growth stage (9).ABA concentration was significantly increased especially at late application (9), when spraying rice plants with 1000 mg L^{-1}. Moreover, JA application recorded a highly decrease in active/inhibitor ratio, compared with untreated plants. Similar findings were reported by Dermastia *et al.* (1994) and Kovac and Ravnikar (1994), who found that, JA application increased cytokinin concentration in potato plants. In this connection, Gendy and Selim (1994) reported that, GA concentration was decreased in faba bean leaves after treated with JA. Moreover, Gendy and Rabie (2000), who showed that, JA application significantly decreased IAA and GA with increasing ABA concentration in Datura leaves.

Yield and its components:

Data in Table (4) showed that grain yield/fed. no of panicles/m^2, 1000 grain weight and starch concentration were increased by JA application. Maximum increment in grain yield/fed.recorded with 500 mg L^{-1} at growth stage 7. The enhancement effect of JA on grain yield may be attributed to increasing number of tillers, starch content, weight of 1000 grains and induction of cytokinin concentration, which led to promoting *Spikelets* and *Floret* development and as a sequence increment of spikelets fertility percentage.

Table (4): Effect of JA on rice yield and its components concentration (combined data of 1999 and 2000 seasons).

JA levels mg.L^{-1}	Growth stage	Grain yield ton/fed	No. of panicls /m^2	1000 grain g	Starch		Protein	
					%	Ton\fed	%	Ton\fed
Control		3.80	350	22.4	56	2.13	13.6	0.517
500	7	4.20	440	23.1	57.2	2.40	14.2	0.596
1000	7	4.25	452	23.5	58.2	2.36	14.6	0.591
500	9	4.10	400	23.0	57.4	2.35	14.0	0.574
1000	9	4.30	442	23.4	58.0	2.50	14.4	0.619
L.S.D 5%		0.44	15	0.15	1.20	_	0.30	_

Regarding to the yield of starch and protein data recorded in Table (4) showed that starch and protein yield were increased by JA treatment. This increase in starch concentration of grains may be due to the capacity of rice plants for CO_2 assimilation and consequently increased the biomass. These results are in full agreement with those reported by Ravnikar *et al.* (1995 a, b) and Gendy and Rabie (2000).

Macroelements concentrations:

a. In shoots:

Nitrogen percentage was increased significantly by JA at high levels, while phosphorus percentage was not affected significantly by JA (Table 5). Potassium percentage was significantly decreased by JA application particularly at growth stage 7. Meanwhile, when rice plants were treated by JA at growth stage 9, recorded increasing in K %.

In this concern, Mengel and Kirkby (1986), reported that root morphology in such as root length, number of root hairs, and probably the individual K^+ uptake. Potential K^+ absorbing power of crop species appear to be important factors influencing competition between plant species for K^+. The reduction of rice shoots with the application of JA was a consequence of increasing root fresh weight.

Table (5): Effect of JA on elements concentration in shoots and grains of rice plant (combined data of 1999 and 2000 seasons).

JA levels mg.L^{-1}	Growth stage	Shoots			Grains		
		N%	P%	K%	N%	P%	K%
Control		3.20	1.0	3.60	2.3	1.60	3.60
500	7	3.30	1.1	3.50	2.4	1.65	3.55
1000	7	3.40	1.3	3.40	2.6	1.75	3.50
500	9	3.35	1.2	3.65	2.5	1.70	3.40
1000	9	3.45	1.4	3.95	2.7	1.80	3.80
L.S.D 5%		0.03	NS	0.03	0.02	NS	0.12

b. In grains:

Data recorded in Table (5) showed clearly that N percentage in rice grains was significantly increased by high dose of JA at both growth stages. The P percentage in grain was not significantly changed by JA treatment. Potassium percentage was significantly increased by JA application.

Improving the minerals percentages in rice shoots and grains as a result of JA may be due to the increasing cytokinins concentration, which led to promoting root morphology and consequently increased macroelements percentages (Ravnikar *et al.*, 1993 and Marshner, 1996).

In conclusion, JA application was more effective at 1000 mg L^{-1} applied at early growth stage (7) especially in reducing rice lodging by increasing plant shortening and increasing tillering which reflected high dry weight, leaf area, and cytokinin concentration, and consequently became higher grain yield.

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تأثر نبات الأرز بمعدلات وميعاد الرش بحمض الجسمونيك

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اجريت تجربتان حقليان بمزرعة كلية الزراعة جامعة المنوفية خلال الموسمين ١٩٩٩ و ٢٠٠٠ وذلك لدراسة تأثير الرش بمعدلات مختلفة من حمض الجسمونيك (صفر و ٥٠٠ و ١٠٠٠ ملليجرام / لـتر) فى ميعادين مختلفين وذلك على نمو محصول نبات الأرز الى جانب الإختلافات فى المكونات الكيميائية.
وقد أمكن إستخلاص النتائج الآتية :

- أدى الرش بحمض الجسمونيك إلى تأثير واضح فى تقليل طول النبات وزيادة عدد الأشطاء وقطر السلاميات حيث أدى الرش المبكر (العمر ٧) إلى معظم التأثير فى نقص الإستطالة.
- أدت المعاملة ١٠٠٠ ملليجرام / لتر عند (العمر ٧) إلى تقصير فى طول السلاميات الوسطى والعليا من الساق.
- وجد ان الرش بحمض الجسمونيك بمعدل ١٠٠٠ ملليجرام / لتر عند العمر ٧ كان مؤثراً فى تقليل الرقاد فى الأرز بواسطة زيادة تقصير النبات وزيادة الأشطاء.
- أدت المعاملة عند (العمر ٧) إلى نقص واضح فى مساحة الأوراق وكذلك نقص فى كمية الكلوروفيل بينما أدت المعاملة عند العمر ٩ إلى زيادة الكلوروفيل.
- أدت المعاملة بحمض الجسمونيك عند العمر ٧ إلى نقص فى تركيز كل من الأندول أسيتيك أسيد والجبريللينات وزيادة فى كل من السيتوكينين وحمض الأبسيسيك.
- كانت الزيادة واضحة فى المحصول ومحتوى الحبوب من النشا والبروتين عند المعاملة بحمض الجسمونيك بتركيز ٥٠٠ ملليجرام / لتر عند العمر ٩.
- أدى الرش بحمض الجسمونيك عند العمر ٩ (قبل طرد السنابل بأسبوعين) إلى زيادة فى محتوى الحبوب من عناصر النيتروجين والفوسفور والبوتاسيوم.
- امكن التوصل من هذا البحث أن معاملة نباتات الارز وخاصة الاصناف الطويلة بحمض الجاسمونيك رشا بتركيز ١٠٠٠ ملليجرام/لتر مبكرا عند العمر (٧) كان مؤثرا في تقليل الرقاد عن طريق زيادة تقصير النبات وزيادة عدد الأشطاء والذي ينعكس على زيادة كل من الوزن الجاف والمساحة الورقية والسيتوكينين والذي ادى إلى زيادة المحصول.